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Research Article

Optimization of Indole-butyric Acid Competency on Root Elongation of *Arecaceae* through Micro-propagation

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Abstract: In the present study optimization of Indole-butyric Acid (IBA) competency on the root elongation was evaluated. The regenerated shoots were separated and shifted to a new media (MS-Basal) containing various concentrations of (IBA) 0.0, 0.5, 1.0, 2.0, 3.0, and 4.0 mg/L and the observations based on the survival rate (%), initiation of roots at a different interval, encountering the total number of roots per shoot and roots length (cm) was noticed. The plants regenerated on (control) MS basal medium where zero concentration of IBA was used, revealed a relatively ($P < 0.05$) lower rate of survival (72.50%) as well as no roots observed till the end of the experiment. While MS-Basal medium supplemented with IBA (2.0mg/L) took minimum time to initiate the roots (16.80 days). The highest number of roots (4.48) appeared in the media containing 3mg/L IBA concentration, whilst 0.5 and 4.0mg/L IBA demonstrated significantly ($P > 0.05$) similar response i.e. 1.83 and 1.70 number of roots respectively, that consequently revealed relatively less number of roots as compared to other concentrations of the media. The MS-Basal media supplemented with 2.0 and 4.0mg/L IBA showed relatively ($P < 0.05$) longer length of roots (2.45 and 2.22 cm) compared to that of MS-Basal media supplemented with 0.5 and 1.0mg/L IBA (1.46 and 1.60 cm, respectively). Besides these things, MS-Basal media supplemented with 3.0 mg/L IBA showed a higher length of roots than the other media.

Keywords: Indole-butyric Acid, Micro-propagation, MS-Basal Medium, Offshoots, Sterilization.

1. INTRODUCTION

The Arecaceae family plants can usually be propagated through seeds, which regularly produce tree-bearing fruits. Root-like branches are preferred for regular reproduction as they produce true-to-type trees of the same fruit [1, 2]. However, there are many problems with this system. The availability of branches is limited because the number produced by each palm tree is very small, unstable, and cannot be successfully controlled [3, 4]. The branches must remain attached to their parent tree for a longer period (2-3 years) until a sufficient root system is developed [5].

The excision method is very complicated and time-consuming, and the percentage of successfully established branches in the soil varies greatly (30 to 80%). The seedlings are variable and it takes 6 to 10 years to grow, while 50% of the seedlings may be males [6, 7, 8]. Also, it has various obstacles like transmitting the disease, scarcity of good quality planting material, and diversification in the yield. On the other hand, in vitro propagation of palm is

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very convenient, because it provides a method to overcome the difficulty of producing maximum numbers of comparatively constant seedlings of the female plants. This system is widely used by many scientists in extensive tissue culture research.

Micro-propagation implicates the production of plants grown up from slight plant fragments, cells, and tissues, particularly in controlled test tubes, where the artificially controlled atmosphere and nourishment are strictly organized. Now, this practice is generally preferable to asexual reproduction in several greenhouse species, because only a minor amount of plant tissue is needed as the initial explant is partly regenerated by millions of clones [8]. In this regard, root stimulating hormone (IBA) performs an important role in the success of regenerative shoot rooting [9]. The increasing global population raising the demand for fruits. The enormous potential of exporting the material along with the farmers hope to cultivate in-vitro propagated banana on a massive scale that can be possible through important explants for rapid propagation and economically important commercial varieties [10, 11]. Considering the importance of propagation through tissue culture the current study was planned whereby the study aimed to determine the effects of different concentrations of IBA on the success of the date palm roots (Fig. 1).

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The offshoots/suckers plants were detached from the mother tree at the age of about 3-4 years from the surrounding areas of the Mirpurkhas and Hyderabad, Sindh. The samples were brought to the Tissue Culture Laboratory, Department of Biotechnology, Faculty of Crop Production, Sindh Agriculture University, Tandojam.

2.2 Preparation of Offshoots/Suckers of Date Palm

The offshoots/suckers of date palm were dissected acropetally and the fibers, dead plant remainders, the basic parts including roots and soil debris, in addition to upper mature parts, were removed (Fig. 2).

2.3 Surface Sterilization

The offshoots were thoroughly cleaned and removed from the outer layer to expose the bud tips and lateral bud areas. The exposed area was removed and directly put in an antioxidant solution (100 mg / L ascorbic acid and 150 mg / L citric acid). The shoot tips and exposed buds were thoroughly sterilized in a 70% solution of ethanol for 15 minutes and then saturated in a 100% sodium hypochlorite inclosing 2 to 3 drops of T-20 for 30 minutes. Besides, the explants were cultured in culturing bottles under a laminar airflow cabinet through flame instruments.

2.4 Preparation of culture medium

The basel medium including micro + macro nutrient + 20 mg/L adenine sulphate, 0.3 g/L activated charcoal, 100 mg/L myoinositol and 0.4 mg/L thiamine HCL supplemented with IBA (0.5-4.0 mg/L), + 30 g/L sucrose and 6 g/L Agar was prepared for root initiation [10] (Supplementary Table S.1).

2.5 Laminar Airflow Cabinet Preparation

Laminar was initiated 30 mins. Formerly the tissue culturing was started. The LAF was sterilized with ultraviolet (UV) rays for 15 min and again thoroughly sterilized with a 70% solution of ethanol. All related instruments such as scissors, tweezers, knives, medium bottles, scalpels, alcohol lamps were kept under a laminar airflow cabinet for proper sterilization.

2.6 Experimental Procedure

The culturing was performed according to the reported shoot tip culture method (Fig. 3) [10, 11]. A total of 30 explants were cultured on basal MS medium containing IBA growth regulator. The excised tips were grown on a basal MS medium containing various concentrations of IBA. In addition, the regenerated shoots/explants were separated and shifted to the rooting medium MS (Basal) + IBA at the supplementation of 0.0, 0.5, 1.0, 2.0, 3.0, and 4.0 mg/L (Supplementary Table S.2). The experiment was repeated three times.

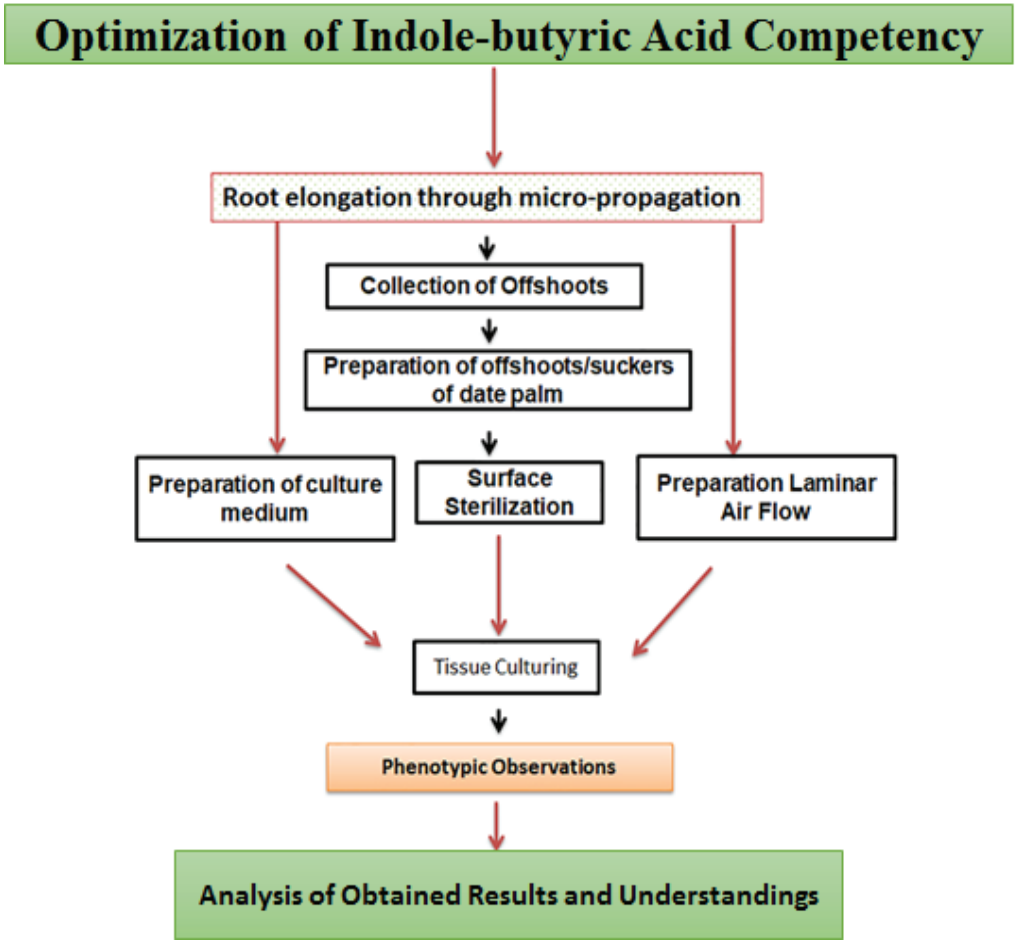


Fig. 1. Research Framework model

Fig. 2. Preparation of offshoot/sucker of the date palm under laboratory conditions. A) Offshoot. B, C) Outer layers removed under laboratory conditions. D) offshoot ready for sterilization and culturing. A total of 20 different offshoots were selected and brought into the laboratory.

Fig. 3. Tissue culturing of the date palm under Laminar Airflow Cabinet. The offshoots were thoroughly sterilized and subjected to culture. The cultured sections of offshoots were transferred to a nutrient medium containing supplementation of BAP and Kinetin.

2.7 Observations

The following characteristics were observed to achieve the objectives of the present study. The survival percentage, days to initiate the roots, encountering the total number of roots, and observing the length of roots (cm).

2.8 Statistical Analysis

The obtained results were statistically analyzed through the method of analysis of variance (ANOVA). The statistical test namely the least significant difference (LSD), was applied to compare the treatments at a 5% probability level. All the calculations and statistical analysis were accomplished by using the student's version 8.1 software package.

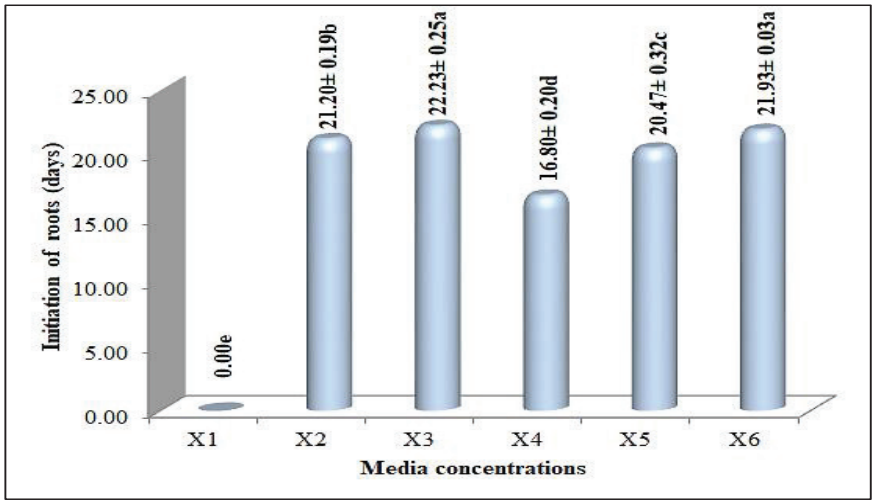
3. RESULTS AND DISCUSSION

3.1 Effect of IBA Competency on the Initiation of Roots

The results for the initial days of buds regenerated and proliferated on MS (basal) medium containing various concentrations of IBA are shown in Fig. 4 and 5. It was found that the shoots regenerated on X3 and X6 media responded similarly and took more time (22.23 ± 0.25 and 21.93 ± 0.03days) to initiate the roots, while the least time was spent on X4 media (16.80±0.20 days). It was further noted that compared to the X2 medium (21.20 ± 0.19 days), the regeneration of date palm roots on the X5 medium (20.47 ± 0.32) took less time. Furthermore, MS (basal) medium did not express any sign of growing up to the end of the experiment (Supplementary Fig.S.1). It was also found that there was no significant ($P > 0.05$) difference observed among the replications of roots regenerated on different media.

3.2 IBA Competency on the Number of Roots

In this study, the effect of nutrient media with different concentrations of IBA was studied, and the results are shown in Fig. 6. It was observed that no roots of date palm had grown on the MS (basal) medium. Whilst, X3, and X4 medium showed comparatively ($P > 0.05$) a similar number of shoots/explants i.e. 2.45 ± 0.09 and 2.3 ± 0.09, respectively. It was also interestingly observed that X2 and X6 media showed more or less relative responses i.e. 1.83 ± 0.76 and 1.70 ± 0.12 number of roots/shoot. At the same time, these media showed significantly fewer roots than date buds regenerated on X3 and X4 media. However, among other mediums, the date buds cultured on



LSD (0.05) = 0.626 SE ± = 0.288 CV% = 2.06

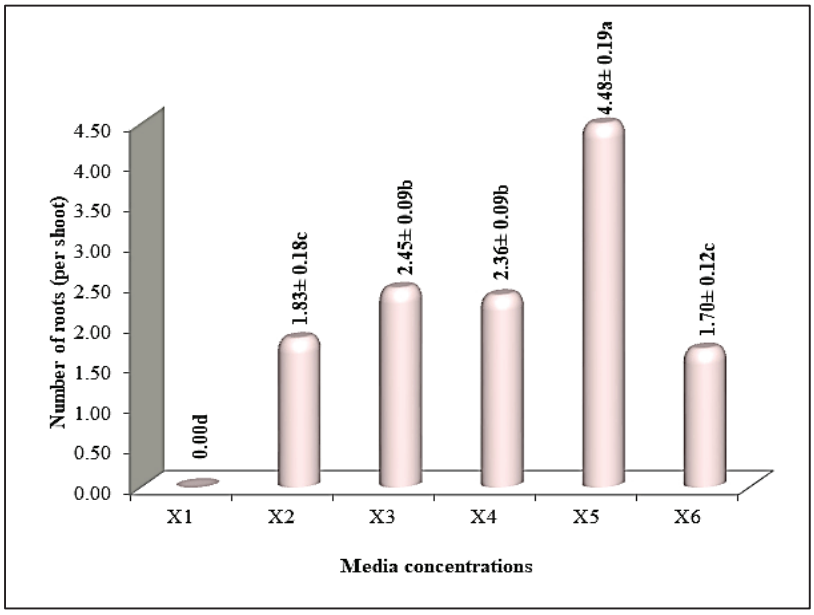
Fig. 4. Initiation of roots (days) of date palm regenerated on MS medium containing different supplementations of IBA. a,b means denotes the statistically significant difference which obtaining through analysis of variance. All means of treatment were compared through LSD at ($P > 0.05$) level of probability. Data are the consequences of three different (biological) replications

the X5 medium showed significantly ($P < 0.05$) the maximum number of roots. It is interesting to note that there was no significant difference observed among the replications of roots regenerated on different media ($P > 0.05$).

3.3 Length of Roots Affected by Different Concentrations of IBA

To further understand the mechanism of in vitro regenerations, the length of roots was determined. The results regarding the length of roots from the shoot of date palm buds regenerated on the MS (basal) medium containing various supplementations of IBA are shown in Fig. 7 and 8. It was surprisingly observed that there were no roots/shoots were formed on the MS basal medium. Whereas, X2 (1.46 ± 0.15 cm) and X3 (1.60 ± 0.15 cm) and/or X4 (2.45 ± 0.18 cm) and X6 (2.22 ± 0.16 cm) exhibited more/less comparable length of roots ($P > 0.05$). However, shoots regenerated on X4 and X6 media exposed significantly ($P < 0.05$)

Fig. 5. Shoots regenerated on MS-Basal medium containing various concentrations of BAP and Kinetin. The results are the consequences of three different biological replicates. The phenotypic observations were taken at two days intervals after initiations.



LSD (0.05) = 0.390 SE ± = 0.179 CV% = 10.26

Fig. 6. Number of roots (per shoot) of date palm regenerated on MS medium containing different supplementation of IBA. [a,b] means denotes the statistically significant difference which obtains through analysis of variance. All means of treatment were compared through LSD at ($P > 0.05$) level of probability. Data are the consequences of three different (biological) replications

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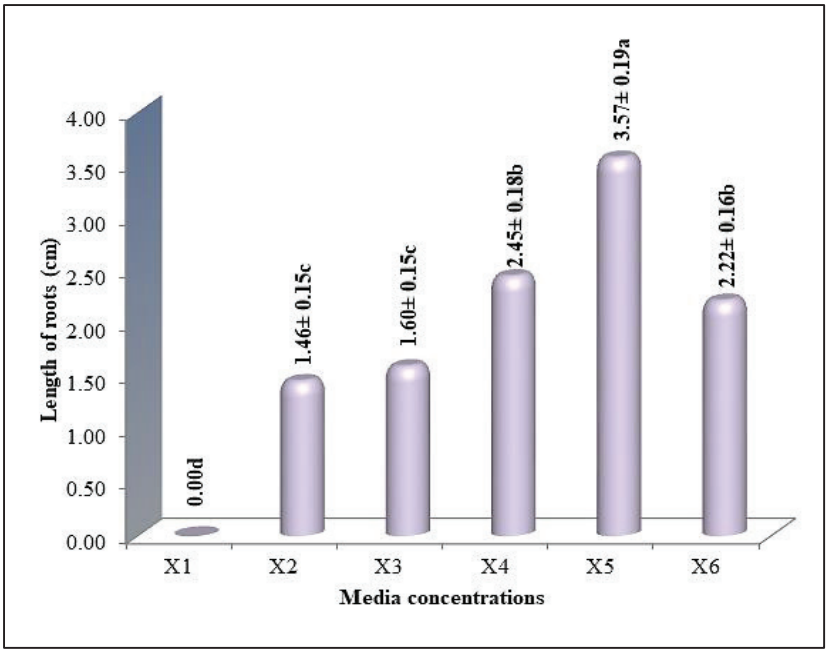

LSD (0.05) =0.465, SE ± = 0.213, CV%= 13.89

Fig. 7. Length of roots (cm) of date palm regenerated on MS medium containing different concentrations of IBA. [a,b] means denotes the statistically significant difference obtained through analysis of variance. All means of treatment were compared through LSD at ($P > 0.05$) level of probability. Data are the consequences of three different (biological) replications

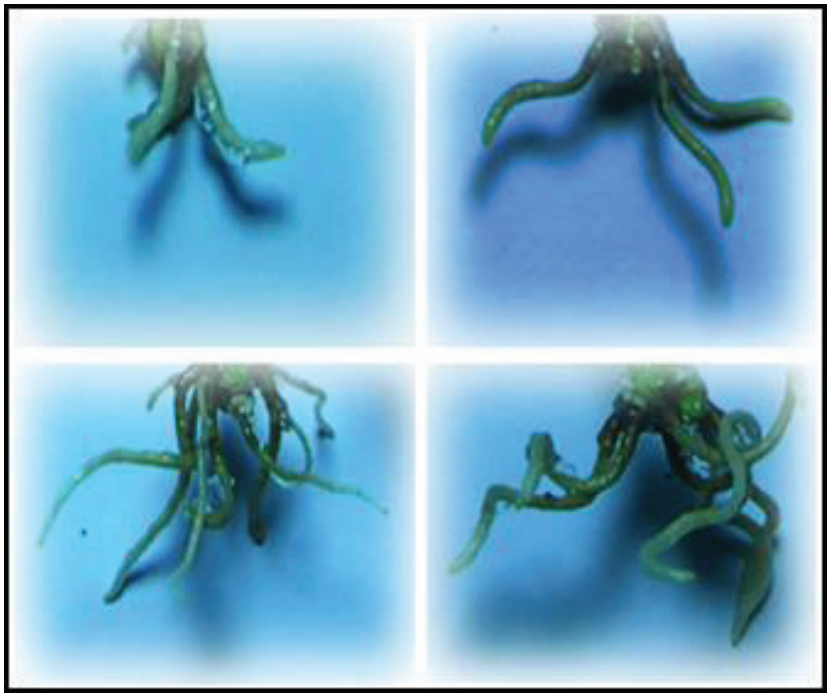

Fig. 8. Roots regenerated on MS-Basal medium containing various supplementations of IBA. Phenotypic results in the consequences of three different biological replicates.

lengthier roots as compared to the shoots exposed to the roots on X2 and X3. Moreover, the X5 medium showed the maximum length of roots i.e. 3.57 ± 0.19 amongst the other media. It was also interestingly noted that there was no significant difference found amongst the various replications of the experiments.

4. DISCUSSION

The tissue culture technology can be used for date palm due to its dioecious characteristics, which limits seed reproduction to produce the plant material. Besides that, the date palm usually does not produce branches, but only single apical tissues of the meristem. It reproduces only a small amount of suction cups early in life so that the number of branches/shoots, and thus the number of meristems tissue used as a source of explants from a single palm is usually small. Although many countries are still improving the protocols to overcome certain growth and reproduction problems, while in some countries, in-vitro regeneration and mass clonal proliferation of date palm has been efficiently used to acquire the maximum quantities of the explants.

4.1 IBA Competency Regulate the Root Regeneration Potential

Compared to other previous reports, this response was quickly terminated [15, 16]. In this regard, if the roots were longer compared to the controlled MS basal, the MS basal medium containing 2.0 mg/L and 4.0 mg/L of IBA showed significantly larger root length ($P < 0.05$) (2.45 and 2.22). The medium supplemented with 0.5 mg/L and 1.0mg/L IBA (1.46 and 1.60, respectively). At the same time, MS basal medium containing 3.0 mg/L IBA showed a longer root length in other mediums. Thus the concluded results are fully supported by the observation of [16, 18, 22]. The slender shoots were cultivated in MS Solid and liquid media with concentrations of 0.0-29.52 µM, 0.0-34.24 µM, and 0.0-32.22 µM, respectively. The above-mentioned researchers noticed a significant difference in the roots at 3.0 mg / L IBA showing clear optimum rooting days (21.20 and 20.47 days, respectively). These results are consistent with the results of different scientists [12, 13, 19, 20]. They noticed the largest root initiation on MS medium supplemented with 24.6 µM IBA. The results on the number of roots showed that the highest number

of roots (4.48) in the medium with a concentration of 3 mg / L IBA. However, MS basal medium containing 0.5mg/L and 4.0mg/L of IBA exhibited comparatively similar response ($P > 0.05$), i.e. 1.83 and 1.70 number of roots. At the same time, these media showed significantly fewer roots than date buds regenerated on the other media. These results are similar to the results [14, 17, 22], and reported that in all tested media, IBA with a concentration of 0.0 mg/L to 6.0 mg/L, there was a significant difference in root induction, root number per branch, and root length response.

5. CONCLUSIONS

The study concludes that the date palm explants cultured on the MS (basal) medium do not show any sign of shoots/explants. The early initiation of roots/shoot occurs at the concentration of 2.0 g/L of IBA, whereas the number and length of roots/ shoot remain maximum on the MS basal medium containing 3.0 mg/L of IBA concentration.

6. ACKNOWLEDGMENTS

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SUPPLEMENTARY DATA

Supplementary Figure S.1. Shoots/roots regenerated on MS-Basal medium supplemented with 0.0 mg/L each of BAP, IBA, and Kinetin (Control). It was noted that there were no roots/shoots were formed in control (without hormones) till the end of the experiment.

Supplementory Table S.2. Composition of Root Induction Media

Media	Concentrations
X1	MS-Basal+0mg/L IBA+ 30g Sugar
X2	MS-Basal+0.5mg/L IBA+ 30g Sugar
X3	MS-Basal+1mg/L IBA+ 30g Sugar
X4	MS-Basal+2.0/L IBA+ 30g Sugar
X5	MS-Basal+3mg/L IBA+ 30g Sugar
X6	MS-Basal+4mg/L IBA+ 30g Sugar

Note:

Combination of Root Induction media

X1 = **MS R**oot-Stimulation-Media I
X2 = **MS R**oot-Stimulation-Media II
X3 = **MS R**oot-Stimulation-Media III
X4 = **MS R**oot-Stimulation-Media IV
X5 = **MS R**oot-Stimulation-Media V
X6 = **MS R**oot-Stimulation-Media VI

Supplementory Table S.2. Composition of MS Basal media

Sr. No.	Ingredients	Quantity used (g/L)
I.	**Micronutrient Stock Solution**	
	Manganese sulphate($MnSO_4.4H_2O$)	16.9
	Zinc sulphate($ZnSO_4.7H_2O$)	08.6
	Boric acid($H_3 BO_3$)	6.2
	Potassium iodide(KI)	0.83
		0.025
	Sodium molybdate($Na MoO_4.2H_2O$)	0.025
	Copper sulphate($CuSO4.5H_2O$)	0.025
	Cobalt chloride($CoCl_2.6H_2O$)	
II.	**Vitamin-based Stock Solution**	
	Pyridoxine HCL($C_8H_{12}ClNO_3$)	0.5
	Thiamine HCL ($C_{12}H_8N_4OSCl_2$)	0.5
	Nicotinic acid ($C_6H_5NO_2$)	0.1
	Glycine (NH_2CH_2COOH)	2.0
	Casein acid	2.0
III.	**Iron Stock Solution**	
	Iron sulphate($FeSO_4.7H_2O$)	27.80
	Sodium EDTA(Na_2 EDTA.$2H_2O$)	37.26

Murashige and Skoog 1962